CHOLINESTERASE IN CEREBROSPINAL FLUID

by

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The notion that acetylcholine (ac. ch.) is of great importance for normal cerebral activity has provoked a careful study of the metabolism of this substance in the brain in health and disease. Obviously investigation of the rate of activity or amount of cholinesterase in brain forms an integral part of this study.

The anticipation that information in this respect could be obtained from a knowledge of the cholinesterase-activity in cerebrospinal fluid (c.s.f.) had led to a number of attempts to assess this activity.

The presence of an acetylcholine destroying factor in c.s.f. was first demonstrated by PLATTNER AND HINTNER[1] and later confirmed by ALTENBURGER[2] and BENDER[3]. These authors used biological methods.

No cholinesterase in c.s.f. could be demonstrated by STEDMAN AND STEDMAN[4] or by VAHLQUIST[5]. With titrimetric methods PINOTTI AND TANFANI[6] could not find evidence for the presence of cholinesterase in c.s.f.; using however a biological method they did demonstrate some cholinesterase-activity which could be inhibited by eserine or by heating. BIRKHÄUSER[7] found cholinesterase in c.s.f. employing a manometric method.

In all the investigations summarized acetylcholine exclusively was used as substrate. As according to the investigations of MENDEL AND RUDNEY[8, 9] and other workers, only true cholinesterase is operative in the metabolism of acetylcholine *in vivo*, it became necessary to extend the preceeding investigations to estimations of cholinesterase-activity in c.s.f. towards "specific" substrates.

GLASSON AND MUTRUX[10] compared the activity of c.s.f. in splitting a non-choline ester (monobutyrine) with the activity towards acetylcholine both with and without eserine in inhibiting concentrations. Low concentrations of eserine, amply sufficient to suppress all cholinesterase-activity, proved unable to inhibit most of the activity of c.s.f. towards monobutyrine from which the presence of an ali-esterase in c.s.f. in addition to true-cholinesterase similar to that in red cells was postulated.

REISS AND HEMPHILL[11] carried out estimations of the cholinesterase-activity in c.s.f. towards *acetyl-β-methylcholine* (amechol), and *benzoylcholine* (benz. ch.) the specific substrates for true- and pseudo-cholinesterase resp.[12]. Using the WARBURG manometric technique they found a fair hydrolysis of acetyl-β-methylcholine, but only a very slight activity towards benz. ch.

Their interpretation was that a considerable part of acetylcholine-hydrolysis by

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c.s.f. could be accounted for by the presence of true cholinesterase. Individual variations in the cholinesterase content of c.s.f. were very marked.

TOWER AND MCEACHERN[13, 14, 15] using similar methods in an extensive investigation confirmed their results. They found no correlation of cerebrospinal cholinesterase value with clinical diagnoses with the exception of cases of craniocerebral trauma and cases treated by electric chock convulsant therapy, where a decrease in specific and a marked increase in unspecified cholinesterase fractions were observed.

The cholinesterase-activity of c.s.f. is very small, approx 1/200 of the cholinesterase activity of an equal volume of blood or serum (10–40 μl/h/ml c.s.f.). The spread is considerable.

Although the presence of small amounts of acetylcholine hydrolysing factors is fairly well established, their quantitative evaluation is still poor, mainly due to technical difficulties encountered in estimating small activities. The results are even less satisfactory when in attempts to differentiate between true- and pseudo-cholinesterase so called "specific" substrates were used, which are less readily hydrolysed than acetylcholine. Very small hydrolyses of "specific" substrates by enzymes for which they are not specific have been demonstrated by ADAMS AND WHITTAKER[16] and these authors point out that it is unwise to draw conclusions concerning the presence of certain enzymes from low hydrolysis values obtained with "specific" substrates. To arrive in spite of these difficulties at reliable information about the specificity and quantity of cholinesterase splitting factors in c.s.f. three different methods of approach were employed:

1. The use of a specific substrate for pseudo-cholinesterase which was more readily hydrolysed than the classical benz. ch.

2. In addition to estimations of the cholinesterase activity towards the recommended "specific" substrates a more complete pattern of esterase activity involving a number of substrates was developed. This was done because, according to NACHMANSOHN[17], such a complete pattern is necessary for accurate assessing of the specificity of cholinesterase-preparations.

3. The activity of c.s.f. was increased by evaporation.

1. The use of butyrylcholine (but. ch.) as a specific substrate for pseudo-cholinesterase.

The low rate of hydrolysis of benz. ch., the substrate usually employed for pseudo-cholinesterase, prompted us to look for a more satisfactory one. It was found that butyrylcholine possessed the following properties (COHEN *et al.*[18]):-

(1) It is a specific substrate for pseudo-cholinesterase.
(2) It is more readily hydrolysed than benz. choline.
(3) It is able to suppress completely the action of true-cholinesterase.

Before it was possible to apply these results obtained with a titrimetric method at 24° C to the estimation of cholinesterase of c.s.f. it was necessary to study the behaviour of but. ch. with the WARBURG-technique at 37.5° C. This technique has to be employed for estimations on c.s.f. because of its low cholinesterase-activity (COHEN *et al.*[19]).

The enzyme preparations employed were ox. nucleus caudatus for the true-cholinesterase*.

* We are indebted to the *Direction of the Public Slaughterhouse* at Leyden for supplies of ox-brain and horse serum.

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The manometric method of AMMON was used at substrate concentrations of 0.016 *M* for acetylcholine, acetyl-β-methylcholine and butyrylcholine. The final volume was 2.5 ml. Enzyme was added from the side bulb after equilibration at 37.5° C. Carbondioxide-development was then followed during 30 minutes. The gaseous medium consisted of N_2/CO_2 5%. Controls were run for autohydrolysis.

It is well known that under the now prevailing conditions pseudo-cholinesterase still efficiently hydrolyses butyrylcholine, whereas it follows from Table I that under these conditions true-cholinesterase is still inactive towards butyrylcholine.

TABLE I

INHIBITION OF THE ACTIVITY OF TRUE CHOLINESTERASE BY BUTYRYLCHOLINE

Exp. No.	Substrate	Activity*	% inhibition
1	Acetylcholine	1836.—	
	Butyrylcholine	0	
	Acetylcholine and Butyrylcholine	556.—	70
2	Acetylcholine	1692.—	
	Butyrylcholine	36.—	
	Acetylcholine and Butyrylcholine	432.—	75
3	Acetylcholine	2117.—	
	Butyrylcholine	0	
	Acetylcholine and Butyrylcholine	756.—	64
4	Acetylcholine	2214.—	
	Butyrylcholine	0	
	Acetylcholine and Butyrylcholine	648.—	71

* Activity in μl CO_2/30 min/ml preparation
Manometric method at 37.5° C

However, concentrations of butyrylcholine (0.016 *M*) which formerly brought about complete inhibition of true-cholinesterase now effected an inhibition of 70% only. This discrepancy could be demonstrated to be due to the difference in temperature during both sets of experiments. Manometric experiments carried out at 24° C (the temperature employed in routine titration experiments) produced practically the same results as the titration experiments previously described, that is: they showed complete inhibition by butyrylcholine (Table II). On the other hand titration experiments performed at 37.5° C resulted in an inhibition of 70% only (Table III).

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TABLE II

INHIBITION OF THE ACTIVITY OF TRUE CHOLINESTERASE BY BUTYRYLCHOLINE

Exp. No.	Substrate	Activity*	% inhibition
1	Acetylcholine	1181.—	
	Acetylcholine and Butyrylcholine	84.—	93
	Butyrylcholine	0	
2	Acetylcholine	1128.—	
	Acetylcholine and Butyrylcholine	43.—	96
	Butyrylcholine	0	
3	Acetylcholine	510.—	
	Acetylcholine and Butyrylcholine	0	100

* Activity in μl CO_2/30 min/ml preparation

Manometric method at 24° C

TABLE III

INHIBITION OF THE ACTIVITY OF TRUE CHOLINESTERASE BY BUTYRYLCHOLINE

Exp. No.	Substrate	Activity*	% inhibition
1	Acetylcholine	5566.—	
	Acetylcholine and Butyrylch. 0.01 *M*	1833.—	68
	Acetylcholine and Butyrylch. 0.02 *M*	1100.—	80
	Acetylcholine and Butyrylch. 0.03 *M*	500.—	91
	Butyrylcholine	0	
2	Acetylcholine	3230.—	
	Acetylcholine and Butyrylch. 0.01 *M*	866.—	73
	Butyrylcholine	0	
3	Acetylcholine	5050.—	
	Acetylcholine and Butyrylch. 0.01 *M*	1300.—	74

* Activity in μl NaOH $\frac{N}{100}$/15 min/ml preparation

Titration method at 37.5° C

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These experiments show a dependence on temperature of the butyrylcholine inhibition. By tripling the substrate concentration the inhibition can be enhanced but even then it does not reach completion.

2. Cholinesterase-activity of c.s.f.

Methods

C.s.f. from patients from various neurological departments* was collected by either lumbar or ventricular puncture and pooled. It was kept in the ice-box at 2° C. After arrival in the laboratory it was spun. The p_H which is usually rather high due to CO_2 loss[20, 21] was adjusted to 7.4.

Enzyme activities were measured manometrically.

C.s.f. (2 ml) was kept in the main compartment of the WARBURG manometer. Substrates were added from the side bulb.

Further conditions were identical to those described in the previous section. Results were expressed as μl CO_2 developed by 2 ml c.s.f. per h.

Spontaneous hydrolysis was allowed for by a correction based on hydrolysis of the substrates concerned in the presence of inactivated (heated or eserine-inhibited) enzyme.

This is necessary because spontaneous hydrolysis in the absence of protein is larger and much more variable. Average values based on a number of spontaneous hydrolyses for various substrates were used for this correction. CO_2 retention was found to be negligible.

Results

The cholinesterase-activities towards the substrates acetylcholine, amechol, benz. choline and butyrylcholine are represented in Table IV.

TABLE IV

HYDROLYSIS OF VARIOUS SUBSTRATES BY POOLED C.S.F.

C.S.F. No.	Activity towards the substrate*			
	Acetyl-choline	Amechol	Benzoyl-choline	Butyryl-choline
1	26	37	12	23
2	82	61	54	67
3	36	14	6	18
4	43	22	11	39
5	53	16	16	53
6	26	13	4	13
7	75	13	11	39
8	62	21	26	50
9	59	22	19	62
10	56	28	18	50
11	47	30	14	38
Average:	51	24	17	41

* Activity in μl CO_2/60 min/2 ml C.S.F.

The average spontaneous hydrolysis of the substrates, calculated from a series of separate experiments, has been allowed for.

* We are indebted to the staffs of the *Ursula Clinic* at Wassenaar, the *Hospital Joannes de Deo* at The Hague and the *Neurological Department of the Academic Hospital* at Leyden for providing us with ample amounts of c.s.f.

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Acetylcholine, amechol, benz. choline and butyrylcholine are all hydrolysed although the activities of different c.s.f.'s vary. The mean values show a clear picture. The order of the rate of hydrolysis is acetylcholine, butyrylcholine, acetyl-β-methylcholine, benz. choline.

These results suggest the presence of true- as well as of pseudo-cholinesterase in c.s.f.

Table V shows a more complete pattern including the hydrolysis of triacetine, ethylbutyrate, propionylcholine, methylbutyrate and tributyrine, obtained for the last five c.s.f.'s of Table IV.

The substrate concentration used was 0.016 *M*.

Triacetine and propionylcholine are known to be attacked by pseudo- as well as by true-cholinesterase, tributyrine by pseudo-cholinesterase and by lipase, ethylbutyrate and methylbutyrate are considered substrates for ali-esterase. Monobutyrine probably holds an intermediate position as a substrate for lipase and ali-esterase.

Table V shows a reasonable hydrolysis by pooled c.s.f. of triacetine, tributyrine and propionylcholine, very little hydrolysis of monobutyrine and no hydrolysis of methyl- and ethylbutyrate.

TABLE V

HYDROLYSIS OF VARIOUS SUBSTRATES BY POOLED C.S.F.

Substrate	7	8	9	10	11
Acetylcholine	75	62	59	56	47
Amechol	13	21	22	18	30
Benzoylcholine	11	26	19	18	14
Butyrylcholine	39	50	62	50	38.5
Triacetine	30	55	53	8?	37
Ethylbutyrate	0	0	9	0	0
Propionylcholine	78	68	90	67.5	55
Methylbutyrate	13	0	27	11	26
Monobutyrine	0	22	5	11	79.5
Tributyrine	—	58	56	54	82.5

Activity in μl CO_2/60 min/2 ml C.S.F.

This pattern is in agreement with the presence of pseudo- and true-cholinesterase which was suggested by the previous results. It clearly shows that no ali-esterase is present and it leaves the question of the presence of a lipase unsolved.

Tributyrine hydrolysis may be due to lipase or to pseudo-cholinesterase. This problem was tackled by first establishing the concentration of eserine which was able to inhibit completely the hydrolysis of acetylcholine.

Table VI shows that this concentration in two experiments was found to be $0.8 \cdot 10^{-4}$ *M* and $0.8 \cdot 10^{-5}$ *M*.

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TABLE VI

INHIBITION OF THE HYDROLYSIS OF ACETYLCHOLINE AND BUTYRYLCHOLINE BY PHYSOSTIGMINE

Exp. No.	Substrate	Activity*	% inhibition
1	Acetylcholine	49	
	Acetylcholine and $0.8 \cdot 10^{-5}$ *M* Physostigmine	4	92
	Acetylcholine and $0.8 \cdot 10^{-4}$ *M* Physostigmine	1	98
	Tributyrine	80	
	Tributyrine and $0.8 \cdot 10^{-4}$ *M* Physostigmine	80	0
	Tributyrine and $0.8 \cdot 10^{-3}$ *M* Physostigmine	76	5
	Tributyrine and $0.8 \cdot 10^{-2}$ *M* Physostigmine	65	9
	Tributyrine and $0.8 \cdot 10^{-1}$ *M* Physostigmine	0	100
2	Acetylcholine	44	
	Acetylcholine and $0.8 \cdot 10^{-4}$ *M* Physostigmine	0	100
	Tributyrine	46	
	Tributyrine and $0.8 \cdot 10^{-4}$ *M* Physostigmine	36	22
	Tributyrine and $0.8 \cdot 10^{-3}$ *M* Physostigmine	26	44
	Tributyrine and $0.8 \cdot 10^{-1}$ *M* Physostigmine	0	100
3	Tributyrine	259	
	Tributyrine and $0.8 \cdot 10^{-4}$ *M* Physostigmine	261	0

Pooled C.S.F. is used in exp. no. 1 and 2; concentrated C.S.F. in exp. no. 3 (Nr. 11, see text).

* Activity in μl CO_2/60 min/2 ml C.S.F.

Tributyrine hydrolysis could only be eliminated by an at least 1000 fold increase in the concentration of eserine.

These findings strongly suggest that at least part of the hydrolysis of tributyrine in pooled c.s.f. is due to the presence of a lipase rather than to a cholinesterase.

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3. *Experiments with concentrated c.s.f.*

The pooled c.s.f.'s of Table IV were evaporated at room temperature *in vacuo* (1 mm Hg) in desiccators over H_2SO_4.

Usually the evaporation was continued until 1/10 of the original volume was reached. This procedure took several days. Once daily the p_H was readjusted to 7.4.

The residue was spun, adjusted to p_H 7.4 and the enzyme activity towards acetylcholine, acetyl-β-methylcholine, benzoylcholine and butyrylcholine was determined.

Activities before and after evaporation are summarized in Table VII. Evaporation of pooled c.s.f. increases usually the activity per ml. The picture is however far from clear. Sometimes (no. 7, 9 en 11) no increase of activity occurs. If it does the rate of increase of activity does not correspond to the rate of concentration (1 : 10) but is considerably less.

TABLE VII

HYDROLYSIS OF VARIOUS SUBSTRATES BY POOLED C.S.F. BEFORE CONCENTRATING (COLUMN A) AND AFTER CONCENTRATING (COLUMN B)

C.S.F. No.	Activity towards the substrate*								
	Acetylcholine		Amechol		Benzoylcholine		Butyrylcholine		
	A	B	A	B	A	B	A	B	
1	26	163	37	71	12	79	23	227	1/5
2	82	224	61	61	54	15	67	168	1/10
3	36	256	14	83	6	22	18	88	1/10
4	43	264	22	28	11	81	39	125	1/10
5	53	184	16	39	16	11	53	53	1/10
6	26	112	13	40	4	15	13	27	1/10
7	75	44	13	12	11	8	38	26	1/10
8	62	231	21	43	26	29	50	238	1/10
9	59	16	12	0	19	0	62	34	1/10
10	56	179	18	38	18	22	50	197	1/10
11	47	17	30	0	14	2	38	8	1/10

The last column indicates to what extent the original volume has been evaporated.

* Activity in μl CO_2/60 min/2 ml C.S.F.

There is also a marked difference in the rate of increase of activity towards various substrates.

This can be seen when the ratio: $\frac{\text{activity conc. c.s.f.}}{\text{activity not conc. c.s.f.}}$ is studied (Table VIII).

This ratio amounts to 4.6 and 4.0 for acetylcholine and butyrylcholine but only to 2.5 and 3.0 for amechol and benz. choline.

Table IX shows a complete pattern of the c.s.f.'s 8 and 10 from Table IV before and after evaporation. The extent of the increase in activity on evaporation varies

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TABLE VIII

VALUES OF THE RATIO $\frac{\text{ACTIVITY CONCENTRATED C.S.F.}}{\text{ACTIVITY NON-CONCENTRATED C.S.F.}}$

C.S.F. No.	Activity towards the substrate			
	Acetyl-choline	Amechol	Benzoyl-choline	Butyryl-choline
1	6.3	1.9	6.5	9.9
2	2.7	1.0	0.3	2.5
3	7.1	5.9	3.7	4.9
4	6.1	1.3	7.4	3.2
5	3.5	2.4	0.7	1.0
6	4.3	0.3	3.7	2.1
7	0.6	0.9	0.7	0.7
8	3.7	2.3	1.1	4.8
9	0.3	0	0	0.5
10	3.2	2.1	1.2	3.9
11	0.4	0	0.1	0.2
Average:	4.6	2.5	3.0	4.0

In calculating the average ratio the values observed in C.S.F. No. 7, 9 and 11 have been omitted, because these C.S.F.'s did not show any increase of activity on evaporation.

TABLE IX

HYDROLYSIS OF VARIOUS SUBSTRATES BY POOLED C.S.F. BEFORE CONCENTRATING (COLUMN A) AND AFTER CONCENTRATING (COLUMN B)

Substrate	C.S.F. No. 8		C.S.F. No. 10	
	A	B	A	B
Acetylcholine	62	231	56	179.5
Amechol	21	43	18	38
Benzoylcholine	26	29	18	22
Butyrylcholine	50	238	50	197
Triacetine	55	246	8?	151
Ethylbutyrate	0	0	0	0
Propionylcholine	68	169	67.5	264
Methylbutyrate	8	9	11	25
Monobutyrine	22	34	11	84
Tributyrine	58	52	54	87

* Activity in μl CO_2/60 min/2 ml C.S.F.

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widely with the substrate used. Activities of c.s.f.'s 9 and 11 (Table VII) estimated in the same way were decreased after evaporation with the exception of the hydrolysis of tributyrine which had increased (56 to 126 and 82 to 259 μl CO_2/60 min/2 ml c.s.f.). This observation provides additional evidence that tributyrine hydrolysis by c.s.f. is mainly effected by the presence of a lipase. Lastly individual c.s.f.'s from lumbar puncture on various patients were studied. Only a limited number of substrates could be used for every c.s.f. The results are given in Table X.

TABLE X

HYDROLYSIS OF VARIOUS SUBSTRATES BY C.S.F. FROM PATIENTS

	Sex	Age	Activity towards the substrate*				Diagnosis
			Acetyl-choline	Amechol	Butyryl-choline	Total-Protein**	
1	♂	43	98		69	26.4	Increased intracranial pressure
2	♀	17	52		29.5	24	Cerebellar process
3	♀	6	36.4		14.2	14	Incipient extrapyramydal syndrom
4	♀	48	52		37	26.4	Syringomyelia
5	♀	59	32.8		27	24	Diabetes insipidus
6	♂	40	20		24	28.8	Sciatica
7	♀	56	46.6		14	26.4	Tabes dorsalis
8	♀	28	20.5		7	19.2	Hernia nuclei pulposi
9	♂	49		33	24	52.8	Tumor cerebri
10	♂	44		27	30	31.2	Disseminated sclerosis
11	♀	56		14.6	25.7	48	Tabes dorsalis
12	♀	16	64	23	43	19.2	Epilepsy
13	♀	40	70	47	25	28.8	Retro-bulbar neuritis
14	♀	3	26	27	30	21.6	Kernicterus
15	♂	45	73	24.5	64	81.6	Tabes dorsalis
16	♂	12	51	24.5	34.5	21.6	Tapetoretinal degeneration
17	♂	36	41	25	35	45.6	Congenital syphilis
18	♂	56	54	20	12	28.8	Tabes dorsalis

* Activity in μl CO_2/60 min/2 ml C.S.F.
** In mg %.

Table XI gives values for individual c.s.f.'s obtained by ventricular puncture towards acetylcholine, amechol, butyrylcholine and tributyrine. These few results are only given as a further demonstration of the presence of true- and pseudo-cholinesterase

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in c.s.f. No attempt is made at this stage to establish relationships between diagnosis and the cholinesterase-content of c.s.f.

Table XI further shows that not every c.s.f. contains a tributyrine hydrolysing factor. This fact strongly suggests that in the c.s.f.'s, which do not hydrolyse tributyrine, pseudo-cholinesterase is not present in sufficient amounts to hydrolyse tributyrine and that lipase is absent. It also provides evidence that when tributyrine hydrolysis does occur it is probably due to a lipase because there is no demonstrable relation between pseudo-cholinesterase and tributyrine splitting activity in different c.s.f.'s.

TABLE XI

HYDROLYSIS OF VARIOUS SUBSTRATES BY C.S.F. OF VENTRICULAR PUNCTURE OF VARIOUS PATIENTS

C.S.F. No.	Activities towards the substrate*				Diagnosis
	Acetyl-choline	Amechol	Butyryl-choline	Tributyrine	
1	64	24	46	15	Occipital neuralgia
2	111	27	67	43	Tumor cerebri
3	74	31	52	0	Epilepsy
4	19	7	21	0	Tumor cerebri

* Activity in μl CO_2/60 min/2 ml C.S.F.

DISCUSSION

The evidence for the presence of cholinesterase, in c.s.f. seems convincing and is mainly based on the following observations:-

1. Pooled c.s.f. as well as individual c.s.f.'s from patients are able to produce a slow but definite hydrolysis of cholinesters.

2. Concentrated c.s.f. is usually able to effect a marked hydrolysis of cholinesters.

3. The hydrolysis of cholinesters can be completely inhibited by eserine ($0.8 \cdot 10^{-4} M$) or by heating at 60° C.

As far as specificity is concerned strong indications exist for the presence of pseudo-cholinesterase.

Evidence is derived from the following results:-

1. Individual c.s.f.'s slowly hydrolyse butyrylcholine.

2. Pooled c.s.f.'s slowly hydrolyse butyrylcholine and benz. choline.

3. Concentrated pooled c.s.f.'s effect a fair hydrolysis of butyrylcholine.

Presence of true-cholinesterase is demonstrated by:-

1. The slow hydrolysis of acetyl-β-methylcholine by individual and pooled c.s.f.'s. The hydrolysis could not be markedly raised by evaporation.

2. Butyrylcholine-hydrolysis in c.s.f. is never greater than acetylcholine-hydrolysis. It has been demonstrated, using human sera, which contain only pseudo-cholinesterase, that the butyrylcholine-hydrolysis is always approximately twice as large as that of acetylcholine. The presence of considerable amounts of true-cholinesterase in c.s.f., relative to its pseudo-cholinesterase-content in contrast to the situation in human serum probably accounts for this difference in behaviour.

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The cholinesterase-content of c.s.f. cannot be attributed to contamination with bloodcells or plasma, because:-

1. The c.s.f.'s used never showed pink decoloration.
2. Practically no red cells were found.
3. The specificity pattern is different from that of blood plasma.

The reasons for the small rate or absence of increase of activity on evaporation are not clear.

The degree of activity resulting from evaporation is never beyond that at which activity and enzyme concentration run parallel. The phenomenon can therefore not be explained simply on the basis of enzyme concentration.

Deterioration of the enzymes present as a result of the treatment may occur. The lack of parallelism between the alteration of activity on evaporation of the same c.s.f. for different substrates may be due to the presence of factors which influence the rate of hydrolysis of various substrates differently at different concentrations.

The presence of inhibiting substances in c.s.f. towards purified preparations is under investigation.

The results of this study confirm those of REISS AND HEMPHILL[11] and TOWER AND MCEACHERN[13, 14, 15] as far as the hydrolysis of acetylcholine, benz. choline and amechol is concerned. Our results do not support the work of GLASSON *et al.*[10], who claimed that c.s.f. contains only true-cholinesterase in addition to ali-esterase. No ali-esterase could be demonstrated in our preparations. Neither do our results agree with their claim that only c.s.f. from patients, suffering from schizophrenia and general paralysis contains pseudo-cholinesterase.

All our c.s.f.'s hydrolysed butyrylcholine and therefore contained pseudo-cholinesterase. Some but not all c.s.f.'s contained lipase.

The material investigated is still too small to allow conclusions on the presence or absence of a correlation between clinical diagnoses and rates of cholinesterase activity in c.s.f.

SUMMARY

1. Butyrylcholine is a specific substrate for pseudo-cholinesterase, and may be used as such for the determination of the pseudo-cholinesterase activity of c.s.f. It is hydrolysed at a faster rate than benzoylcholine.

2. Butyrylcholine is an inhibitor of true-cholinesterase.

3. Individual c.s.f.'s of human patients hydrolyse acetylcholine, butyrylcholine and acetyl-β-methylcholine and sometimes tributyrine.

4. Pooled c.s.f.'s from a number of human patients hydrolyse acetylcholine, acetyl-β-methylcholine, benzoylcholine, propionylcholine, butyrylcholine, triacetine, tributyrine and sometimes monobutyrine. No hydrolysis of ethyl- or methylbutyrate could be demonstrated.

5. The weak cholinesterase-activity of c.s.f. may be enhanced by evaporation. The increase of activity per ml by this procedure is less than would be predicted from the degree of concentration. It moreover differs with the substrates used.

6. Tributyrine hydrolysis may be inhibited by eserine, but only in concentrations which are at least 1000 times stronger than those, necessary for the complete inhibition of acetylcholine hydrolysis.

7. It is concluded that c.s.f. contains pseudo- as well as true-cholinesterase. Sometimes lipase is present. Ali-esterase does not occur in c.s.f.

RÉSUMÉ

1. La butyrylcholine est un substrat spécifique de la pseudo-cholinestérase et peut être employée pour la détermination de l'activité pseudocholinestérasique du liquide cérébro-spinal (l.c.s.). Elle est hydrolysée plus rapidement que la benzoylcholine.

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2. La butyrylcholine est un inhibiteur de la cholinestérase vraie.

3. Des l.c.s. de patients individuels hydrolysent l'acétylcholine, la butyrylcholine et l'acétyl-β-methylcholine et parfois la tributyrine.

4. Des l.c.s. recueillis de plusieurs patients hydrolysent l'acétylcholine, l'acétyl-β-methylcholine, la benzoylcholine, la propionylcholine, la butyrylcholine, la triacetine, la tributyrine et parfois la monobutyrine. Nous n'avons pas pu mettre en évidence l'hydrolyse d'éthyl- ou de méthylbutyrate.

5. L'activité cholinestérasique faible du l.c.s. peut être augmentée par évaporation. L'augmentation d'activité ainsi obtenue par ml est plus faible que la concentration permettrait de prévoir. De plus, elle varie avec le substrat employé.

6. L'hydrolyse de la tributyrine peut être empêchée par l'ésérine, mais seulement à des concentrations mille fois supérieures à celles qui empêchent complètement l'hydrolyse de l'acétylcholine.

7. Nous concluons de ce qui précède que le liquide cérébro-spinal contient aussi bien de la pseudo- que de la vraie cholinestérase et parfois de la lipase, mais pas d'ali-estérase.

ZUSAMMENFASSUNG

1. Butyrylcholin ist ein spezifisches Substrat für Pseudocholinesterase und kann zur Bestimmung der Pseudocholinesterase-Aktivität in der Cerebrospinalflüssigkeit dienen. Butyrylcholin wird schneller hydrolysiert als Benzoylcholin.

2. Butyrylcholin ist ein Hemmstoff für wahre Cholinesterase.

3. Cerebrospinalflüssigkeit (C.S.F.) von einzelnen Patienten hydrolysiert Acetylcholin, Butyrylcholin und Acetyl-β-methylcholin und manchmal Tributyrin.

4. Gesammelte C.S.F. von einer Anzahl von Patienten hydrolysiert Acetylcholin, Acetyl-β-methylcholin, Benzoylcholin, Propionylcholin, Butyrylcholin, Triacetin, Tributyrin und manchmal Monobutyrin. Eine hydrolytische Wirkung auf Äthyl- oder Methylbutyrat konnte nicht nachgewiesen werden.

5. Die schwache Cholinesterase-Aktivität der C.S.F. kann durch Eindampfen erhöht werden, aber die Aktivitätszunahme pro ml ist bei diesem Verfahren geringer als auf Grund der Konzentration zu erwarten wäre, und hängt ausserdem von dem verwendeten Substrat ab.

6. Die Hydrolyse von Tributyrin kann durch Eserin gehemmt werden, aber nur wenn die Konzentration mindestens 1000 mal grosser ist, als diejenige, welche zur vollständigen Hemmung der Acetylcholinhydrolyse notwendig ist.

7. Die Verfasser schliessen, dass die C.S.F. sowohl Pseudo wie wahre Cholinesterase enthält. Manchmal ist Lipase anwesend. Ali-esterase kommt in der C.S.F. nicht vor.

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